Evaluation of Hepatitis C Virus Core Antigen Assays in Detecting Recombinant Viral Antigens of Various Genotypes[▽]

Mohsan Saeed,[1,3] Ryosuke Suzuki,[1] Madoka Kondo,[1] Hideki Aizaki,[1] Takanobu Kato,[1]
Toshiaki Mizuochi,[2] Takaji Wakita,[1] Haruo Watanabe,[1,3] and Tetsuro Suzuki[1*]

*Department of Virology II[1] and Department of Safety Research on Blood and Biological Products,[2] National Institute of
Infectious Diseases, Tokyo 162-8640, and Department of Infection and Pathology, Graduate School of
Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033,[3] Japan*

**A single substitution within the hepatitis C virus core antigen sequence, A48T, which is observed in ~30%
of individuals infected with genotype 2a virus, reduces the sensitivity of a commonly used chemiluminescence
enzyme immunoassay. Quantitation of the antigen is improved by using a distinct anticore antibody with a
different epitope.**

Hepatitis C virus (HCV) is a major cause of chronic liver disease throughout the world. Accurate diagnosis of HCV infection is important due to the morbidity associated with the virus, and determining the level of viral replication is important in predicting and monitoring the effect of antiviral treatment. Although quantifying viral RNA represents the standard method for identifying active infection (5, 8, 13), several sensitive immunoassays that detect the viral core antigen (Ag) have now been developed as an alternative to HCV RNA testing (3, 4, 6, 9, 10, 12, 16). The amino acid sequence of the core Ag is largely conserved among different viral isolates (14); however, genetic variability of the virus constitutes one of the major challenges to using core Ag assays for diagnosis. In this study, we examined the effects of sequence heterogeneity on the sensitivity of diagnostic kits for detection of the core Ag by using recombinant Ag derived from each of the major HCV genotypes. Expression plasmids for epitope-tagged core Ag were generated by inserting cDNA for the full-length core region of genotype 1a (17; GenBank accession no. AF011751), 1b (1; D89815), 2a (7; AB047639), 2b (AB030907), or 3a virus, with a FLAG tag sequence attached at its 5′ end, into the EcoRI site of the pCAG mammalian expression vector (11). HEK293T cells transiently transfected with the expression plasmids were harvested 48 h after transfection using a passive lysis buffer (Promega, Madison, WI). Centrifugation was performed to remove the debris after ultrasonication. Total protein was quantified in aliquots of cell lysate by using the bicinchoninic acid method (Pierce, Rockford, IL) and then used for determining the concentrations of HCV core Ag.

Figure 1A shows comparable levels of core Ag in each sample of cell lysate, as determined by immunoblotting with anti-FLAG antibody (Ab). The ability of HCV core Ag assays to detect five different HCV genotypes were compared using a commercially available chemiluminescence enzyme immuno-

assay (CLEIA) (Lumipulse II HCV core assay [assay detection range, approximately 50 to 50,000 fmol/liter]; Fujirebio, Japan) (15) and enzyme-linked immunosorbent assay (ELISA) (Ortho HCV Ag ELISA test [assay detection range, approximately 44.4 to 3,600 fmol/liter]; Ortho-Clinical Diagnostics, Japan) (2) to detect HCV core Ag in cell lysate. As shown in Fig. 1B,
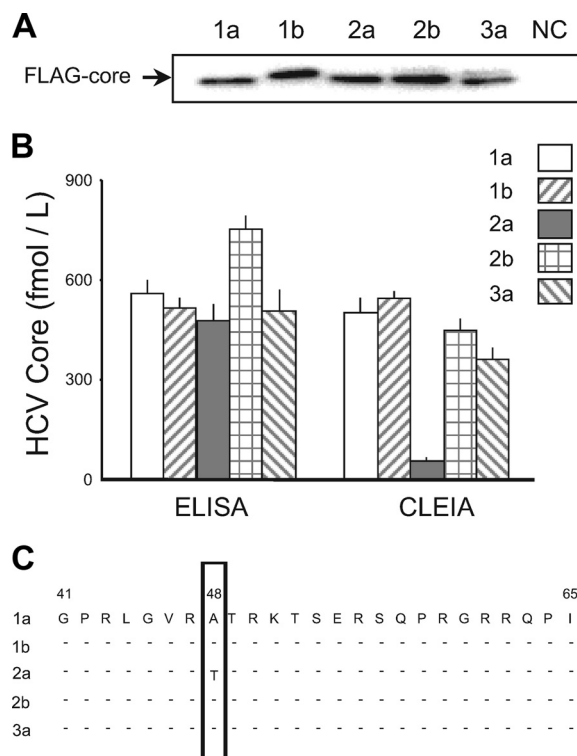

FIG. 1. Detection of recombinant HCV core Ag derived from genotype 1a, 1b, 2a, 2b, and 3a isolates by immunoblotting using an anti-FLAG Ab (A) as well as ELISA and CLEIA (B). The data shown in panel B represent the mean values and standard deviations (*n* = 3). NC, negative control. (C) The amino acid sequence from amino acids 41 to 65 of the core Ag used in this study. Key residues at the 48th position are boxed. Hyphens indicate conservation.

* Corresponding author. Mailing address: Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Phone: 81-3-5285-1111. Fax: 81-3-5285-1161. E-mail: tesuzuki@nih.go.jp.

TABLE 1. Comparison of the 48th residues of HCV core Ags of genotypes 1a, 1b, 2a, 2b, and 3a

Genotype	No. of isolates	No. (%) of isolates with residue at 48th position		
		T	A	Other
1a	263	9 (3.5)	254 (96.5)	0 (0)
1b	298	2 (0.7)	294 (98.6)	2 (0.7)
2a	17	5 (29.5)	12 (70.5)	0 (0)
2b	17	0 (0)	17 (100)	0 (0)
3a	23	0 (0)	23 (100)	0 (0)
Total	618	16 (2.6)	600 (97.1)	2 (0.3)

although the ELISA measured similar concentrations of core Ag in all samples, apparent low levels of the genotype 2a core Ag, originally from an isolate known as the JFH-1 isolate (7), were detected using the CLEIA method, suggesting that some differences in the amino acid sequences corresponding to particular HCV genotypes or isolates may influence the sensitivity of core Ag detection. A comparison of the core Ag sequences, including the monoclonal Ab epitopes used in the development of CLEIA, revealed conservation of alanine at the 48th position in four clones, of genotypes 1a, 1b, 2b, and 3a, but not genotype 2a, for which there is a threonine at this position (Fig. 1C). Based on our analysis of sequences available from the HCV database (http://hcv.lanl.gov/content/sequence/NEWALIGN/align.html), alanine is highly conserved at the 48th residue of the core Ag for HCV isolates of genotypes 1a, 1b, 2b, and 3a (Table 1). In contrast, alanine and threonine occur in this position in 70.5% and 29.5%, respectively, of genotype 2a isolates. To examine whether the low sensitivity of the CLEIA method might be due to this particular amino acid change, we next replaced threonine with alanine at the 48th position of the JFH-1 core Ag (for
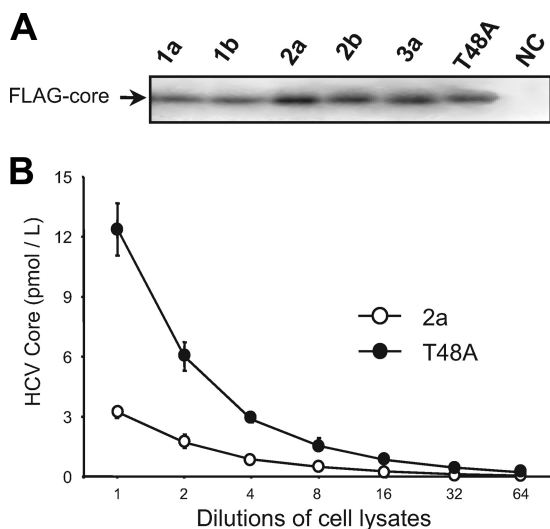

FIG. 2. Effect of T48A substitution in the core Ag of the JFH-1 isolate with regard to sensitivity of the CLEIA method. Samples of wild-type or mutated core Ag cell lysate were analyzed by immunoblotting (A) and CLEIA (B). The data shown in panel B represent the mean values and standard deviations ($n = 3$). NC, negative control.

TABLE 2. Comparison of the modified CLEIA with the original version for detection of the core Ags of genotypes 1a, 1b, 2a, 2b, and 3a[a]

Genotype	CLEIA	HCV core antigen concn (fmol/liter) in serially diluted cell lysates at indicated fold dilution							
		1	2	4	8	16	32	64	128
1a	Original	11,147	5,527	2,611	1,484	691	403	195	101
	Modified	10,511	5,700	2,676	1,420	716	444	200	111
1b	Original	11,612	5,618	3,081	1,551	779	409	223	113
	Modified	11,192	6,028	2,824	1,522	804	431	197	101
2a	Original	3,216	1,710	844	480	232	104	48	36
	Modified	12,101	6,255	3,153	1,676	805	422	212	106
2b	Original	10,559	5,635	2,811	1,286	762	387	194	94
	Modified	10,977	6,179	3,381	1,624	842	437	219	129
3a	Original	11,478	5,891	2,922	1,414	756	422	212	112
	Modified	11,208	6,225	3,126	1,555	791	445	215	100

[a] Data represent the mean values in triplicate measurements.

the mutant JFH-1coreT48A) and measured the HCV core Ag concentration in cells expressing both mutated and wild-type JFH-1 core Ag. After confirming comparable levels of FLAG-tagged core Ag in the cell lysate samples by immunoblotting (Fig. 2A), HCV core Ag was quantified in the samples by serial dilution via the CLEIA method. As shown in Fig. 2B, the core Ag concentrations of JFH-1coreT48A were assessed to be 3.2- to 3.8-fold higher than those of the wild-type core Ag, suggesting that the sensitivity of HCV core Ag detection may have been affected by the 48th residue in the core Ag. Data for samples derived from genotypes 1a, 1b, 2b, and 3a were analogous to data for JFH-1coreT48A (data not shown). Although HCV isolates with threonine at the 48th position of the core Ag sequence comprise a relatively small proportion of the major genotype population, only 2.6% of the genotype 1a, 1b, 2a, 2b, and 3a isolates here (16 of 618 isolates; Table 1), attempts to overcome this problem would improve the overall sensitivity and usefulness of the assay. To achieve this aim, another monoclonal anticore Ab, whose epitope is comprised of amino acids 50 to 65, which are completely conserved among all the genotypes examined (Fig. 1C), was therefore used as a second Ab in a modified version of the CLEIA. We compared this modified assay with the original version by measurement of core Ag concentrations of the various genotypes (Fig. 2A) as illustrated in Table 2. The modified assay was able to quantify core Ag from genotypes 1a, 1b, 2a, 2b, and 3a with no significant differences observed between Ag levels in samples from different genotypes at each dilution.

It has been demonstrated that the HCV core Ag assay is a useful alternative to HCV RNA quantification for the diagnosis of hepatitis C and for monitoring the antiviral effects of treatment. Compared to various reverse transcription-PCR methods, HCV core assays are less expensive and easier to perform, without the requirement of sophisticated laboratory equipment and specially trained laboratory personnel. In addition, the core Ag assay can be used to measure a more diverse set of blood samples, such as sera stored for a long period of time, because the viral Ag is generally more stable than the RNA in sera or plasma. Despite the adequate performance of core Ag assays, we have shown that a single amino acid substitution at the 48th position of the core Ag changes the detection sensitivity. It is also noted that, although the original CLEIA should be improved, the ELISA used in this study may be substituted for it.

In conclusion, we have identified a distinct anticore Ab with a different epitope that might enable improved detection across all of the major HCV isolates. The findings of this study would provide useful information for the development of an improved assay with greater accuracy.

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